10.1117/2.1201211.004595

Photoacoustic microscopy using Förster resonance energy transfer

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High-resolution images in deep tissue are obtained using photoacoustic images of Förster resonance energy transfer, overcoming the problem of light-scattering effects in some imaging techniques.

Förster resonance energy transfer (FRET) is a physical process in which energy is transferred from an excited donor fluorophore to adjacent chromophores non-radiatively.[1] Because the transfer rate is sensitive to the distance between donor and acceptor, FRET provides a molecular 'ruler' for measuring the distance between biomolecules. The process is valuable to the understanding of protein interactions and conformational changes, and we can use it, in vivo, to explore protease activity, protein misfolding, and intracellular calcium.

FRET manifests through the reduction of donor fluorescence emission as a result of non-radiative energy transfer to the acceptor, so fluorescence imaging is well suited to produce FRET images. However, the intensive light scattering that occurs beyond the depth of a few hundred microns precludes fluorescence imaging of FRET at greater depths and with high spatial resolution.

We have developed high-resolution photoacoustic microscopy of FRET in deep tissue.[2] Photoacoustic imaging is based on detecting non-radiative decay processes ultrasonically. When a fluorophore is excited, it undergoes either transitions of fluorescence emission, or it experiences fast non-radiative decay to the ground state. FRET provides a third mechanism for excited state decay, where energy is transferred from a donor fluorophore to an acceptor chromophore by non-radiative dipole-dipole coupling. If a non-fluorescent acceptor is chosen, all the transferred energy must decay through the acceptor's non-radiative pathway. The non-radiative decay converts the excited molecular state energy into heat and subsequent thermoelastic expansion, generating acoustic waves (photoacoustic signals) in the medium. Therefore, when

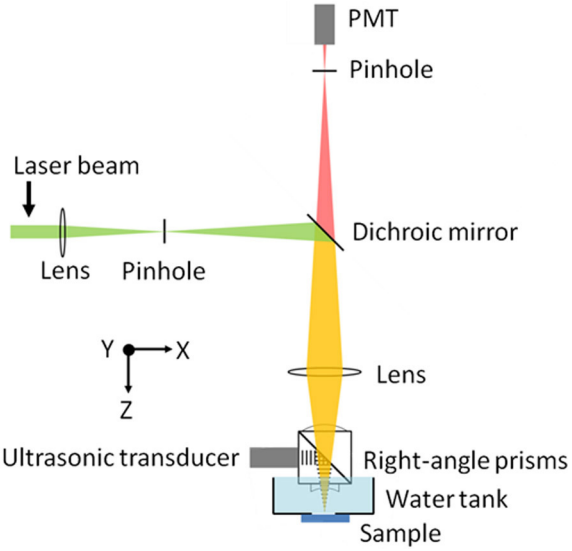

Figure 1. *Schematic diagram of a dual-modality fluorescence and photoacoustic microscope used to demonstrate acoustic imaging of Förster resonance energy transfer (FRET). PMT: Photomultiplier.*

FRET occurs, an increase of photoacoustic signal strength accompanies the donor fluorescence quenching. While fluorescence imaging observes a decrease in donor fluorescence or an increase in acceptor fluorescence, photoacoustic imaging visualizes the increase in pressure generated by the acceptor. Photoacoustic imaging enables deep penetration because the magnitude of acoustic scattering in tissue is three times less than that of optical scattering. Moreover, photoacoustic imaging is scalable with optical illumination and ultrasonic detection. It can provide submicron resolution at a depth of 1cm while maintaining a high depth-to-resolution ratio.[3]

We demonstrated photoacoustic imaging of FRET in solutions using a dual-modality photoacoustic and fluorescence confocal microscope (see Figure 1).[4] We prepared seven stock ethanol solutions with concentrations of fluorescent donor Rhodamine

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6G (R6G) and non-fluorescent acceptor 1,3 'diethyl-4,2' quinolyoxacarbocyanide iodide (DQOCI), as in Figure 2(a). Figure 2(b) and (c) shows the fluorescence and photoacoustic images acquired at 523nm. When the donor R6G was excited, we detected fluorescence and photoacoustic emissions in the presence of different concentrations of acceptor DQOCI. Compared with a solution containing only donor R6G (tube 1), the mixtures containing both donor R6G and acceptor DQOCI had diminished fluorescence signals. Increasing the quantity of acceptor DQOCI made fluorescence quenching more effective, and it led to a higher photoacoustic signal (tubes 3, 5 and 7). The FRET efficiency mappings based on the fluorescence and photoacoustic measurements are shown in Figure 2(d) and (e), respectively. The color-coded images display the increase in FRET efficiency due to the increase in the acceptor concentration.

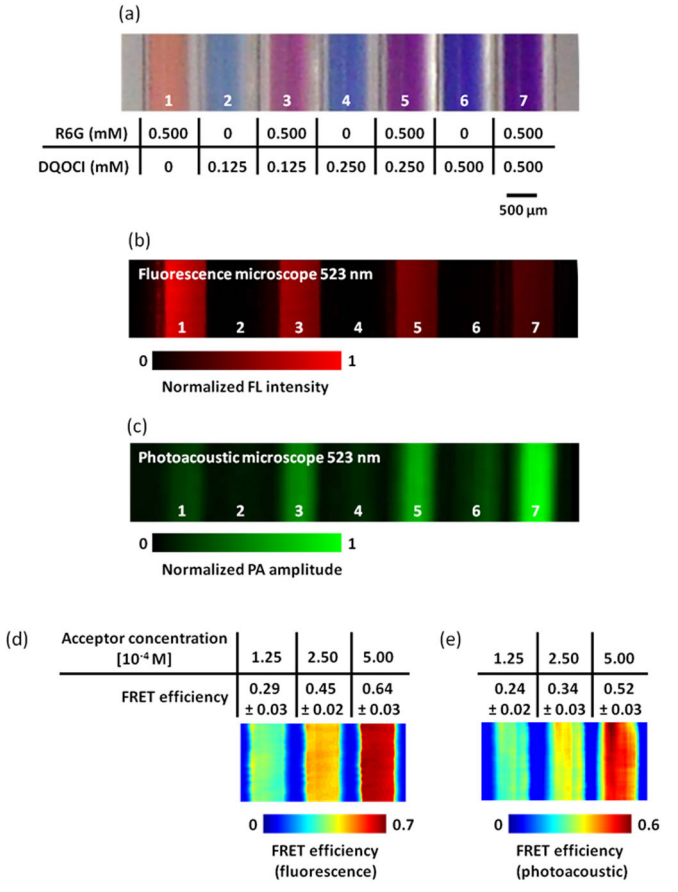

Figure 2. *(a) Photograph of the tube phantom and table showing concentrations of donor Rhodamine 6G (R6G) and acceptor DQOCI. (b) Fluorescence microscopic image acquired at 523nm. (c) Photoacoustic microscopic image acquired at 523nm. (d) FRET efficiency maps acquired using the fluorescence microscope. (e) FRET efficiency maps acquired using the photoacoustic microscope.*

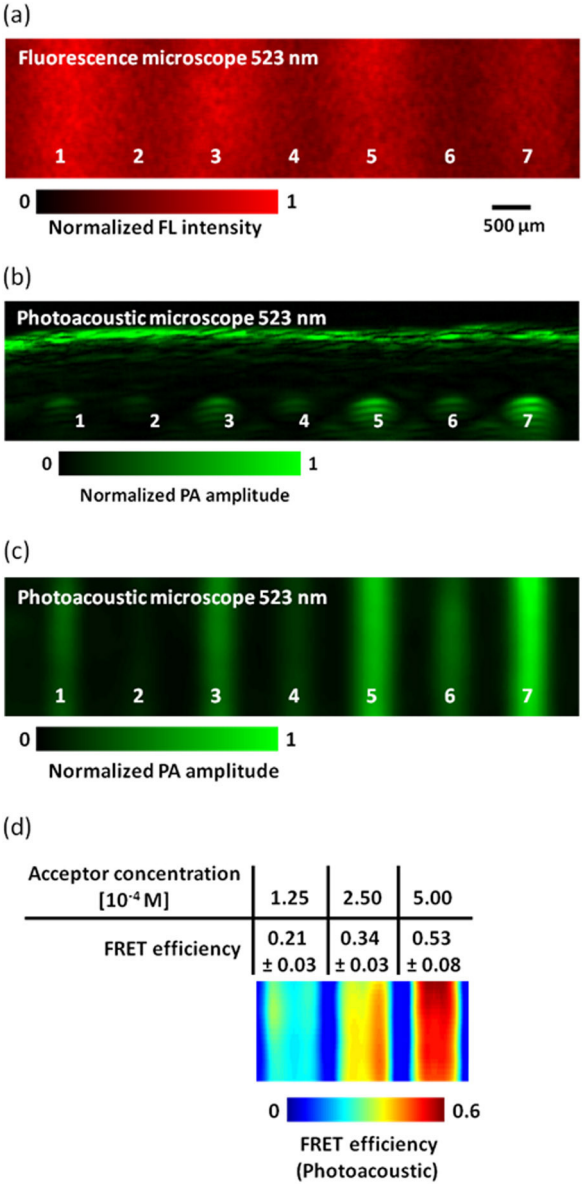

Figure 3. *(a) Fluorescence microscopic image, acquired at 523nm, of the tube phantom with overlaid mouse skin tissue. (b) Cross-sectional B-scan photoacoustic microscopic image of the tubes acquired at 523nm. (c) Photoacoustic microscopic image acquired at 523nm. (d) FRET efficiency map acquired using the photoacoustic microscope.*

To compare the photoacoustic and fluorescence approaches for deep tissue FRET imaging, we overlaid intervening mouse skin tissue on the samples. The tubes detected in the fluorescence confocal image were severely blurred by light scattering in the tissue: see Figure 3(a). The photoacoustic cross sectional B-scan image of the tubes shows that the overlaid mouse skin

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had a thickness of ∼1mm: see Figure 3(b). Again, the photoacoustic images of Figure 3(c) show FRET quenching of the donor R6G fluorescence by acceptor DQOCI. Photoacoustic microscopy achieves FRET imaging of the tissue phantom, as in Figure 3(d), whereas we cannot generate a similar map from fluorescence microscopy because of its poor signal-to-noise ratio: see Figure 3(a).

Photoacoustic microscopy has been used to image FRET efficiencies through a 1mm skin tissue, and further study has demonstrated a penetration of 10mm in scattering tissue. Based on the relative increase of photoacoustic signals, we can quantify absolute FRET efficiency. Compared with confocal microscopy, photoacoustic microscopy offers better penetration into scattering biological tissue. In our future work, we will extend photoacoustic FRET imaging to in vivo animal studies.

This work was sponsored in part by National Institute of Health grants R01 EB000712, R01 EB008085, R01 CA134539, U54 CA136398, R01 CA157277, and R01 CA159959. Lihong Wang has a financial interest in Microphotoacoustics Inc. and Endra Inc., although these companies did not support this work.

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